Development of an ADC Process with Single-Use Membrane Technology

Jieyu Zhou*[1], Jesse Novak[1], Juan Carlos Cordova[1], Sheng Sun[1], Jeffrey Bos[1], Srinath Thirumalairajan[1], Lake N. Paul[1], Sanjeevani Ghone*[1], Miyako Hirai[2], Ricarda A. Busse[2], Ian Schwartz[3]
[1] Abzena, 360 George Patterson Blvd. Bristol, PA 19007, United States
[2] Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen, Germany
[3] Sartorius Stedim North America, 5 Orville Drive, Suite 200. Bohemia, NY 11716, USA
* Jieyu.zhou@abzena.com and sanjeevani.ghone@abzena.com

Abstract

Membrane chromatography is routinely used to remove host cell proteins, viral particles, and aggregates during antibody downstream processing. The application of membrane chromatography to the field of antibody-drug conjugates (ADCs) has been applied in a limited capacity and in only specialized scenarios.

Here, we utilized the characteristics of the membrane adsorbers, Sartobind® S and Phenyl, for aggregate and payload clearance whilst polishing the ADC in a single chromatographic run. The Sartobind® S membrane was used in the removal of excess payload while the Sartobind® Phenyl was used to polish the ADC by clearance of unwanted DAR species and aggregates. The Sartobind® S membrane reproducibly achieved log-fold clearance of free payload with a ten membrane volume wash. Application of the Sartobind® Phenyl decreased aggregates and higher DAR species while increasing DAR homogeneity. The Sartobind® S and Phenyl membranes were placed in tandem to simplify the process in a single chromatographic run. With the optimized binding, washing and elution conditions, the tandem membrane approach was performed in a shorter timescale with minimum solvent consumption with high yield. The application of the tandem membrane chromatography system presents a novel and efficient purification scheme that can be realized during ADC manufacturing.

Introduction

The development of cation-exchange and hydrophobic interaction membrane chromatography for site-specific ADC process is described using an engineered cysteine-mAb with Pyrrolobenzodiazepine (PBD)-dimer as a model conjugation system. Key process parameters such as product yield, efficiency of free PBD-dimer and aggregate removal were evaluated.

Process Diagrams

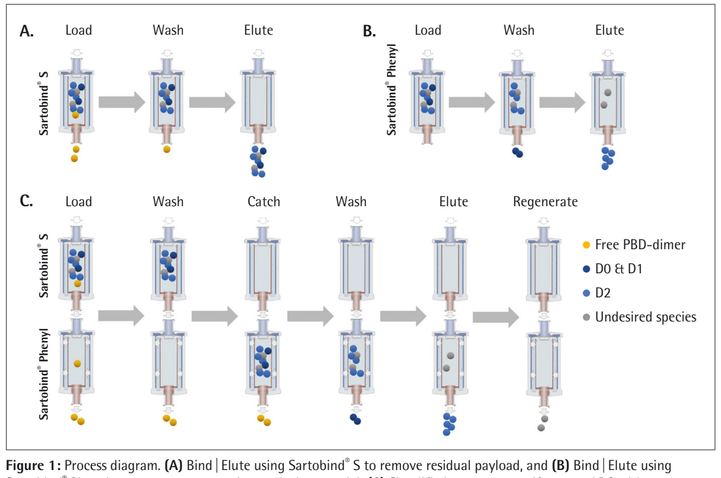

Figure 1: Process diagram. **(A)** Bind | Elute using Sartobind® S to remove residual payload, and **(B)** Bind | Elute using Sartobind® Phenyl to remove aggregate in stand-alone model; **(C)** Simplified process to purify target ADC with Sartobind® S and Phenyl in tandem model.

Results

Sartobind® S DBC

mAb: 32 mg/mv
MMAE-ADC: 37 mg/mv
PBD-ADC: 34 mg/mv

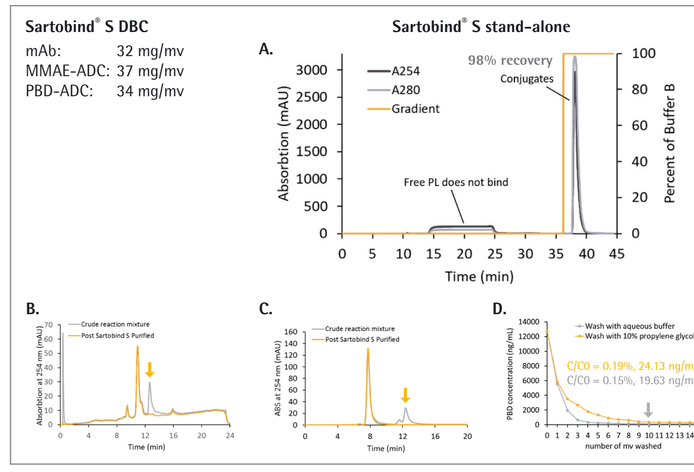

Figure 2: (A) Removal of residual PBD using Sartobind® S in stand-alone model. The quenched reaction mixture (gram scale) was loaded to a 150 mL Sartobind® S membrane, then washed with 10 mv binding buffer prior to elute. **(B)** HIC and **(C)** SEC profiles showing the removal of free PBD dimer. **(D)** LC-MS method showing PBD dimer removal efficiency with Sartobind® S.

Sartobind® Phenyl DBC

mAb: 13 mg/mv
MMAE-ADC: 14 mg/mv
PBD-ADC: 14.9 mg/mv

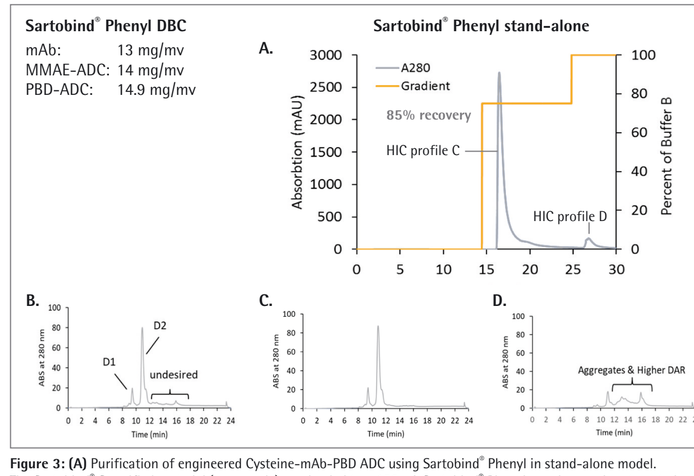

Figure 3: (A) Purification of engineered Cysteine-mAb-PBD ADC using Sartobind® Phenyl in stand-alone model. The Sartobind® S purified material (gram scale) was loaded to a 150 mL Sartobind® Phenyl membrane, then targeted and undesired ADC species were eluted sequentially. **(B)** HIC profile of Sartobind® S purified ADC. **(C)** HIC profile of Sartobind® Phenyl purified ADC. **(D)** Aggregate and higher DAR species was in the final wash step.

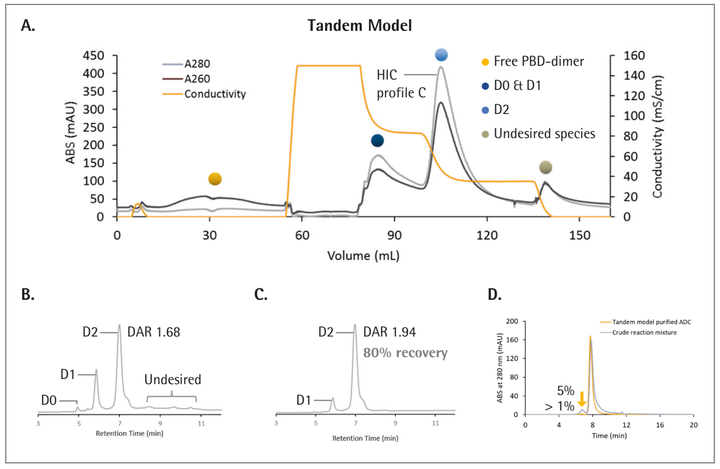

Figure 4: (A) Purification of engineered Cysteine-mAb-PBD ADC in tandem model. The quenched reaction mixture (gram scale) was loaded to Sartobind® S which was tandemly connected to Sartobind® Phenyl. The conjugation species were separated through loading, washing, and multiple elution steps. **(B)** HIC profile showing the crude reaction mixture contains ADC variants with a range of DAR, residual PBD dimer, aggregates, and organic solvent. **(C)** HIC profile of the tandem method purified ADC. The DAR of the ADC increased from 1.68 to 1.94. **(D)** SEC profile showing the percentage of aggregate dropped from 5% to less than 1% after tandem model purification.

Conclusions

- Membrane based process consumed less buffer, shortened process and hold times (less GMP scientist FTE days) which can reduce the cost and time for a cGMP manufacturing campaign.
- Membrane devices are scalable single-use, closed systems that improve manufacturing safety, eliminating the need of packing, qualification, and cleaning validation studies associated with resin-based column chromatography.
- Removal of free payload and undesired conjugate species by membrane chromatography presents a novel and efficient process that directly translates into improved efficiency both during process development and cGMP manufacturing.